

Appl. No. 09/622,613
Supplemental Response dated January 16, 2004
Reply to Office Action of July 3, 2003

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No new claim amendments are presented. This listing of claims replaces all prior versions in the application.

Listing of Claims:

1. (original) A recombinant ribonuclease that has (a) measurable ribonuclease activity; (b) an amino terminal end beginning with a glutamine; (c) a leucine at position 11; an asparagine at position 21, a threonine at position 85, and a histidine at position 103, such positions being determined through alignment with reference to those specified amino acid positions of SEQ ID NO:2; and (d) substantial identity to SEQ ID NO:2.
2. (original) The recombinant ribonuclease of claim 1, further comprising a methionine residue at position 1.
3. (original) The recombinant ribonuclease of claim 2, wherein the methionine residue at position 23 as shown in SEQ ID NO:2 is replaced with a leucine residue.
4. (original) The recombinant ribonuclease of claim 3, further comprising histidine residues at 1 through 6 (SEQ ID NO:9).
5. (original) The recombinant ribonuclease of 1, wherein the glutamine at position 1 is cyclized to pyroglutamic acid.
6. (original) The recombinant ribonuclease of claim 1, wherein the glutamine at position 1 is replaced with a serine.
7. (original) A cytotoxic reagent comprising the recombinant ribonuclease of claim 1, linked to a ligand binding moiety.

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8. (original) The cytotoxic reagent of claim 7, further comprising a methionine residue at position 1.
9. (original) The cytotoxic reagent of claim 8, wherein the methionine residue at position 23 as shown in SEQ ID NO:2 is replaced with a leucine residue.
10. (original) The cytotoxic reagent of claim 9, further comprising histidine residues at 1 through 6.
11. (original) The cytotoxic reagent of claim 7, wherein the glutamine at position 1 is cyclized to pyroglutamic acid.
12. (original) The cytotoxic reagent of claim 7, wherein the glutamine at position 1 is replaced with a serine.
13. (original) The cytotoxic reagent of claim 7, wherein the ribonuclease of SEQ ID NO:2 is linked to a ligand binding moiety through a covalent bond.
14. (original) The cytotoxic reagent of claim 13, wherein said covalent bond is at the carboxy terminus of the ribonuclease of SEQ ID NO:2.
15. (original) The cytotoxic reagent of claim 7, wherein said ligand binding moiety is an antibody directed against a cell surface antigen present on a cancer cell.
16. (original) The cytotoxic reagent of claim 15, wherein said antibody is a recombinant single chain antibody.

Claims 17-19 (cancelled)

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20. (withdrawn) A nucleic acid which encodes a recombinant ribonuclease having a nucleotide sequence as shown in SEQ ID NO:14 and conservative variants thereof.
21. (withdrawn) A recombinant ribonuclease encoded by a nucleic acid comprising SEQ ID NO:14 and conservative variants thereof.
22. (withdrawn) The ribonuclease of claim 21, wherein the amino acid sequence is selected from the group consisting of SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26.
23. (withdrawn) A cytotoxic reagent comprising the ribonuclease of claim 22 linked to a ligand binding moiety.
24. (withdrawn) The cytotoxic reagent of claim 23, wherein the ribonuclease is linked to a ligand binding moiety through a covalent bond.
25. (withdrawn) The cytotoxic reagent of claim 24, wherein said covalent bond is at the carboxy terminus of the ribonuclease.
26. (withdrawn) The cytotoxic reagent of claim 23, wherein said ligand binding moiety is an antibody directed against a cell surface antigen present on a cancer cell.
27. (withdrawn) The cytotoxic reagent of claim 26, wherein said antibody is a recombinant single chain antibody.

Claims 28-30 (cancelled)

31. (withdrawn) A method of preparing a substantially pure recombinant ribonuclease, said method comprising:

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(i) contacting said ribonuclease with an effective concentration of a cleaving agent such that the ribonuclease is cleaved after the carboxy group of methionine at position 1;

(ii) passing said ribonuclease through a Ni^{2+} -NTA agarose column;

and

(iii) eluting said substantially pure ribonuclease from said column.

32. (withdrawn) A method of preparing a substantially pure recombinant cytotoxic reagent, said method comprising:

(i) contacting said cytotoxic reagent with an effective concentration of a cleaving agent such that the cytotoxic reagent is cleaved after the carboxy group of methionine at position 1;

(ii) passing the cytotoxic reagent through a Ni^{2+} -NTA agarose column;

and

(iii) eluting said substantially pure cytotoxic reagent from said column.

33. (withdrawn) The method of claim 31, 32, wherein said cleaving agent is CNBr.

34. (original) A pharmaceutical composition comprising a ribonuclease expressed from recombinant DNA, said ribonuclease comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24, and SEQ ID NO:26 in a pharmaceutically acceptable carrier.

35. (previously presented) The pharmaceutical composition of claim 34, further comprising an antineoplastic.

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36. (previously presented) The pharmaceutical composition of claim 35, where said antineoplastic is Adriamycin.

37. (original) A pharmaceutical composition comprising a cytotoxic reagent, said cytotoxic reagent comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24 and SEQ ID NO:26 in a pharmaceutically acceptable carrier.

38. (original) A method of killing cancer cells comprising contacting cells to be killed with a ribonuclease expressed by recombinant DNA and having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24 and SEQ ID NO:26.

39. (original) A method of killing cancer cells comprising contacting cells to be killed with a cytotoxic reagent expressed by recombinant DNA, comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:24 and SEQ ID NO:26 covalently linked to a ligand binding moiety, said ligand binding moiety directed against a cell surface antigen on the cancer cells.

41. (original) The method of claim 39, wherein said ligand binding moiety is an antibody.

42. (original) The method of claim 41, wherein said antibody is a single chain antibody.